The Examiner asserts that Nanbu et al. and Uenoyama et al. both disclose surfactants used in bioassays, and that surfactants improve assay sensitivity. The Examiner further states that Nanbu et al. and Uenoyama et al. teach the equivalence of polyoxyethylene sorbitan monolaurate and polyoxyethylene sorbitan monooleate to sucrose monolaurate surfactants (Nanbu et al.) and n-octyl-B-D-thioglucoside (Uenoyama et al.).

Therefore, the Examiner takes the position that it would have been obvious to substitute the surfactants of Nanbu et al. or Uenoyama et al. for the surfactant of Chu, because the references teach that surfactants improve assay sensitivity, and because the references show equivalence among the surfactants.

Applicants' Remarks

1. *The use of a surfactant in an immunochromotography device is old in the art*

Both Chu and the present application relate to the use of a surface active agent in an immunochromatography device. It is known to add a surface active agent to a reaction membrane, and to dry the surface active agent in an immunochromatography device which employs a porous reaction membrane on which at least a receptor is immobilized. ("Drying" the surface active agent means evaporating moisture components if the surface active agent is in a liquid state at normal temperature.) For example, Japanese published patent application No. 62-71861 (filed in 1986, and described in Applicants' specification) teaches Tween20 (polyoxyethylene sorbitan monolaurate) and TritonX100 (t-Octylphenoxypolyethoxyethanol) (both known surface active agents) as reagents which are used in an immune reaction component measuring method.

Further, using a porous carrier as a reaction layer, and employing an antibody as a reaction component is disclosed in Japanese Published Patent Application No. 11-153601 (filed September 18, 1997, and also described in Applicants' specification). This device is an extracorporeal medicament or a portable diagnostic device which is intended to detect microorganisms in water or to detect a minute amount of marker substances from bodily fluid. More particularly, this device is an immunochromatography device which is used in a small-sized clinic which has no analysis equipment, or which is used by a person being tested. JP '601 teaches a nonionic surface

active agent in an alkylphenolether system, and further teaches that a surface active agent of the triton system, particularly TritonX-100, is preferred.

It is clear from the above-discussion that the technique of employing a surface active agent in an immunochromatography device was well known to those of ordinary skill in the art, prior to the filing of the Chu application. However, neither Tween20 nor TritonX100, which are disclosed in JP '861 and JP '601, are surface active agents which comprise a sugar in a hydrophilic part and are solidified when dried. [As discussed in the Response After Final Rejection, filed April 5, 2005, a surface active agent which is solidified when dried is a surface active agent which is a solid at normal temperature and normal pressure.]

Both Tween20 and TritonX100 are also disclosed in the laundry list of surface active agents in the Chu reference. However, as admitted by the Examiner, Chu does not describe the use of a surface active agent which comprises a sugar in a hydrophilic part.

2. *The advantages of employing a surface active agent which is solidified when dried and comprises a sugar in a hydrophilic part*

By using a surface active agent which is solidified when dried, the devitalization of the reactive component immobilized on the reactive layer can be minimized, thereby realizing enhanced preservation stability, extended quality maintenance period, and expanded storage condition of the chromatography medium. (See page 7, lines 14-19 of Applicants' specification.) Further, by using a surface active agent which also comprises a sugar in a hydrophilic part, in addition to the advantages discussed above, the solubility is enhanced and the permeability is increased by the action of the sugar. Additionally, influence on the protein can be reduced and the denaturization and devitalization of the immobilized protein can be minimized, therefore extending the performance of the reactive layer for a long time. (See page 9, lines 1-11 of Applicants' specification.)

On the contrary, in a conventional immunochromatography device, a surface active agent is generally employed only for improving permeability of the reaction layer. When a surface active agent which comprises no sugar in its hydrophilic part, and which is in a liquid state or in a paste-like state at normal temperature and normal pressure is employed, it is impossible to dry the surface active agent to an absolutely dry condition. This leads to gradually advanced devitalization of the immobilized antibody during the preservation period of the immunochromatography device, thereby deteriorating the performance of the material. This

in turn unfavorably shortens the quality preservation period of the material as well as restricts the storage condition of the material. Therefore, when a surface active agent which comprises no sugar in its hydrophilic part and which is in a liquid state or in a paste-like state at normal temperature and normal pressure is employed, it is impossible to provide a material with the high precision and high preservation stability of Applicants' claimed chromatography medium.

3. *Remarks traversing the obviousness of combining the teachings of Chu and Nanbu et al.*

The invention of Nanbu et al. relates to a method for measuring the concentration or activity of urinary trypsin inhibitor (UTI). Nanbu et al. teach that a sample, a protease, a substrate and a surface active agent are mixed, and the enzyme activity of the protease (enzyme) is measured. Nanbu et al. disclose three measuring methods, including one which does not employ a surface active agent at all. Further, Nanbu et al. describe that it is unclear why the protease activity is improved by adding the surfactant, but the improvement is significant. (See column 2, lines 54-56 of Nanbu et al.) Additionally, Nanbu et al. disclose a method that is obtained by combining the method that does not include a surface active agent and the method that does include a surface active agent. Nanbu et al. teach that this third method has extremely high sensitivity. (See column 3, lines 11-13 of Nanbu et al.)

Nanbu et al. also disclose that "[o]ne of the characteristics of the present invention is to use a surfactant. The surfactant has no limitation in kind. Any ionic surfactant, ampholytic surfactant, or nonionic surfactant may be used. The optimum range of the amount to be used is also determined suitably depending on the kind of surfactant." (Emphasis added) (See column 5, lines 32-37 of Nanbu et al.) Nanbu et al. also describe that a preferable surfactant is selected from the group consisting of polyoxyethylene sorbitan monolaurate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monostearate, sorbitan monopalmitate, sorbitan monooleate, sorbitan monolaurate, polyoxyethylene (10) octylphenylether, polyoxyethylene (30) octylphenylether, polyoxyethylene (40) octylphenylether, 1-O-n-octyl-ß-D-glucopyranoside, sucrose monolaurate, 3-[(3-cholamido propyl)dimethylammonio]-propanesulfonic acid, 3-[(3-cholamido propyl)dimethylammonio]-2-hydroxypropanesulfonic acid,

cetyltrimethylammonium bromide and benzyltrimethylammonium hydroxide." (See column 3, lines 26-38 of Nanbu et al.) This laundry list of preferred surface active agents includes all types of surfactants: ionic surfactants, ampholytic surfactants and nonionic surfactants. Therefore, the invention of Nanbu et al. does not depend on the kind of the surface active agent. Nanbu et al. do not teach or suggest choosing a surface active agent which comprises sugar in a hydrophilic part and which is solidifed when dried, as required by Applicants' claim 1. On the contrary, Nanbu et al. clearly state that there is no limitation, whatsoever, on the surface active agent to be used. This is clearly different from Applicants' claimed invention, which requires a particular surface active agent in order to achieve the advantages discussed in part 2. above. The Examiner has provided no reason why one of ordinary skill in the art would select a particular surface active agent from the list provided in Nanbu et al., nor why one of ordinary skill in the art would select a surface active agent that is solidified when dried and comprises a sugar in a hydrophilic part.

MPEP 2143.02 states that the prior art can be modified or combined to reject claims as obvious as long as there is a reasonable expectation of success. *In re Merck & Co., Inc.*, 231 USPQ 375 (1986). Further, the MPEP states that at least some degree of predictability is required. As discussed above, both Chu and Nanbu et al. disclose a laundry list of possible surface active agents. The Examiner asserts that it would be obvious to substitute one particular surface active agent of Nanbu et al., drawn from a disclosure which permits the use of any surface active agent, for the surface active agent of Chu. However, as discussed in part 2. above, using a surface active agent that is solidified when dried and comprises a sugar in a hydrophilic part results in advantages which are not present when a surface active agent comprising no sugar in a hydrophilic part, and which is in a liquid state or paste-like state at normal temperature and pressure, is used. Neither Chu nor Nanbu et al. discuss the advantages obtained by employing this particular type of surfactant. Therefore, it is untenable to assert that there is any degree of predictability when "choosing" one of the many surface active agents to substitute for the surface active agent of Chu.

There is no teaching or suggestion in the combination of Chu in view of Nanbu et al. to choose the particular surface active agent recited in Applicants' claims. Further,

there is no teaching or suggestion regarding the advantages obtained when using Applicants' particularly recited surface active agent. For these reasons, the rejection based on Chu in view of Nanbu et al. is unfounded.

4. *Remarks traversing the obviousness of combining the teachings of Chu and Uenoyama et al.*

The invention of Uenoyama et al. relates to a method for measuring the concentration of unitary trypsin inhibitor (UTI) and a method of dissolving its substrate. Uenoyama et al. disclose arranging and mixing a sample, protease, calcium, and substrate, and measuring the enzyme activity of the protease to assay the protease inhibitor in the sample.

Uenoyama et al. disclose that the conventional methods prior to their invention had the following problems. When the calcium concentration mixed in the buffer solution is low, trypsin may be activated by the influence of calcium present in the urine sample, so that the observed trypsin activity measurement would indicate a lower value for the UTI concentration than the real value. (See column 1, lines 39-46 of Uenoyama et al.)

Further, when an excess amount of calcium is added, it reacts with carbonate ions, phosphate ions and the like, all of which are present in the urine, thereby producing precipitates which will unfavorably affect the measurement. Although a pretreatment such as centrifugation may be conducted in order to remove them, this may complicate the measurement. (See column 1, lines 46-51 of Uenoyama et al.)

Further, an organic solvent such as DMSO may damage a plastic cell which is generally used in an automatic analytical apparatus, so that the amount of the organic solvent which can be used is limited. Accordingly, the amount of the substrate which can be dissolved in the organic solvent is also limited. As a result, the sensitivity of the measurement becomes difficult to improve and the reproducibility is limited. (See column 1, lines 52-56 of Uenoyama et al.)

Moreover, there is a possibility of trypsin activity being inhibited by using an organic solvent. In addition, the rather insoluble BAPNA can be dissolved by using an organic solvent, but if the amount used is not sufficient, there is a possibility of BAPNA crystallizing out of solution when the substrate solution is kept in long-term storage or in refrigeration. Therefore, in a conventional measuring method, when a slightly soluble substrate

such as BAPNA is used with an organic solvent, it is necessary to adjust the substrate solution for each measurement, and then carry out the measurement immediately. (See column 1, lines 58 to column 2, line 2 of Uenoyama et al.)

The method of dissolving the substrate of Uenoyama et al. is dissolving the substrate in an organic solvent and diluting this solution by water. This method adds at least one surface active agent which is an ampholytic surfactant or a nonionic surfactant to at least one of the organic solvent and water. (See column 5, lines 13-19 of Uenoyama et al.)

Uenoyama et al. disclose that a betaine type amphoteric surfactant is preferably used as the surfactant. Uenoyama et al. disclose that as the amphoteric surfactant, at least one of 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid and 3-[(3-cholamidopropyl)dimethylammonio]-2- hydroxy-1-propanesulfonic acid is preferably used. Further, Uenoyama et al. teach that as the nonionic surfactant, at least selected from the group consisting of polyoxyethylene sorbitan monolaurate, polyoxyethylene sorbitan monooleate, polyoxyethylene(23)lauryl ether, polyoxyethylene(20)cetyl ether, polyoxyethylene(10)octylphenyl ether, polyoxyethylene nonylphenyl ether, polyoxyethylene alkyl ether, perfluoroalkyl polyoxyethylene ethanol, alkylester fluoride, polyethylene glycol mono-p-nonylphenylether, polyoxyethylene(30)octylphenyl ether, N,N-bis (3-D-gluconamidopropyl) deoxycholamide, n-octyl- β–d thoiglucoside and sucrose monolaurate is preferably used. Uenoyama et al. teach Noigen EA-80, Noigen EA-120, and Noigen EA-140 (all products of Daiichi Kogyo Seiyaku Co. Ltd.) as examples of polyoxyethylene nonylphenyl ether; Softanol 70, Softanol 90, and Softanol 120 (all products of Nippon Syokubai Co., Ltd.) as examples of polyoxyethylene alkyl ether; Fluorad FC-170C (Product of 3M) as an example of perfluoroalkyl polyoxyethylene ethanol; Fluorad FC-430 (Product of 3M) as an example of alkylester fluoride; and TRITON X-305 (Product of Nacalaitesque) as an example of polyoxyethylene (30) octylphenyl ether. (See column 3, line 54 to column 4, line 14.)

While Uenoyama et al. do not clearly teach the effect and mechanism of the invention, it is believed that by using specified surfactants in diluting, the amount of organic solvent (which adversely affects the enzyme activity of trypsin) can be reduced. Additionally, a sufficient amount of substrate can be used, and the enzyme activity of trypsin increases to a value higher than normal. Thereby, the

measurement precision and within-run reproducibility of the measurement can be improved. In a situation where a reaction system includes insoluble BAPNA as a substrate, and an organic solvent, such as DMSO, is employed, and a plastic cell is damaged by the organic solvent to affect the measurement, the problem arising in such a situation is solved by the addition of a particular surface active agent.

The Examiner asserts that since Uenoyama et al. teach that the use of the surface active agent enhances the measurement sensitivity, it would be obvious for one skilled in the art to use the surface active agent that is shown in Uenoyama et al. in the construction of Chu. However, the reaction system of Uenoyama et al. is limited to include protease, insoluble substrate, organic solvent, plastic, and the like. In addition, in view of the goal of enhancing the measurement sensitivity in the reaction system, the addition amount of the substrate is regulated because the substrate is insoluble in water. When the substrate concentration is increased, the substrate is precipitated in the reaction system, thereby unfavorably affecting the measurement. Further, while an organic solvent having a solvability higher than that of water is employed in Uenoyama et al. in order to dissolve an insolvable substrate, this deteriorates the plastic cell in the reaction, and unfavorably affects the measurement. Uenoyama et al. teach that these problems can be solved by using a surface active agent, specifically:

1) in the process of diluting an insoluble substrate, the solvability of the insoluble substrate is enhanced, and the substrate addition amount is increased, thereby enhancing the measurement sensitivity; and

2) by reducing the amount of the organic solvent, damage to the plastic cell in the reaction are reduced, and the measurement sensitivity is thus enhanced.

On the contrary, the present invention has a construction where:

1-1) no insoluble substrate is included,

1-2) there is no process of diluting the insoluble substrate, and

2-1) no organic solvent is employed,

2-2) the reaction system does not comprise a plastic cell, and

2-3) therefore there can be no damage to the plastic cell.

Therefore, the present invention is very different from Uenoyama et al. in its all aspects.

Uenoyama et al. disclose making the insoluble substrate soluble by adding the surface active agent. Contrary to Uenoyama et al., the present invention does not include an insoluble substance, plastic cell, or organic solvent

Furthermore, Applicants have discovered that among 16 kinds of surface active agents which are disclosed in Uenoyama et al. as an ampholytic surfactant or a nonionic surfactant, only those which are solidified when dried and comprise sugar in a hydrophilic part, such as n-octyl--13-d-thioglucoside and sucrose monolaurate, are particularly superior surface active agents. On the contrary, there is no teaching or suggestion in Uenoyama et al. to choose this particular type of surface active agent from those disclosed by the reference. The Examiner has provided no reason why one of ordinary skill in the art would select a particular surface active agent from those disclosed in Uenoyama et al., nor why one of ordinary skill in the art would select a surface active agent that is solidified when dried and comprises a sugar in a hydrophilic part. Nor do Uenoyama et al. discuss that one type of surfactant has an advantage over the others present in the list.

As discussed above regarding Chu in view of Nanbu et al., MPEP 2143.02 states that the prior art can be modified or combined to reject claims as obvious as long as there is a reasonable expectation of success. *In re Merck & Co., Inc.*, 231 USPQ 375 (1986). Further, the MPEP states that at least some degree of predictability is required. As discussed above, both Chu and Uenoyama et al. disclose a laundry list of possible surface active agents. The Examiner asserts that it would be obvious to substitute one particular surface active agent of Uenoyama et al., drawn from a disclosure which permits the use of any surface active agent selected from the disclosed list, for the surface active agent of Chu. However, as discussed in part 2. above, using a surface active agent that is solidifed when dried and comprises a sugar in a hydrophilic part results in advantages which are not present when a surface active agent comprising no sugar in a hydrophilic part, and which is in a liquid state or paste-like state at normal temperature and pressure is used. Neither Chu nor Uenoyama et al. discuss the advantages obtained by employing this particular type of surfactant. Therefore, it is untenable to assert that there is any degree of predictability when "choosing" one of the many surface active agents to substitute for the surface active agent of Chu.

There is no teaching or suggestion in the combination of Chu in view of

Uenoyama et al. to choose the particular surface active agent recited in Applicants' claims. Further, there is no teaching or suggestion regarding the advantages obtained when using Applicants' particularly recited surface active agent. For these reasons, the rejection based on Chu in view of Uenoyama al. is unfounded and should be withdrawn.

5. *Conclusion*

In summary, the inventors of the present invention have discovered that by employing a surface active agent that is solidified when dried and comprises a sugar in its hydrophilic part in an immunochromatography measurement apparatus,

1) due to the function of sugar, late solvability is enhanced, and the permeability of the reaction layer is enhanced, and

2) the transformation or deactivation of an immobilized specific protein can be suppressed to the minimum extent, and the reaction layer can be preserved for a long period of time.

Based on Applicants' claimed invention, enhancement of the preservation stability, lengthening of the quality preservation period, and relaxation of the maintenance condition of the chromatography specimen become possible.

For the reasons stated above, the subject matter of claims claims 5, 12, 27, 31, 41, 45, 53 and 60 is clearly patentable over Chu in view of Nanbu et al. or Uenoyama et al.

The rejection of claim 49 under 35 U.S.C. § 103(a) as being unpatentable over Chu in view of Nanbu et al. or Uenoyama et al., and further in view of Iwata et al. is respectfully traversed.

The comments set forth above are equally applicable to this rejection. Since claim 45 is directly dependent on claim 12, the subject matter of claim 45 is patentable over Chu in view of Nanbu et al. or Uenoyama et al. for the same reasons that the subject matter of claim 12 is patentable over this combination of references. The teachings of Iwata et al. do not remedy the deficiencies of these references.

Therefore, in view of the above remarks, it is submitted that each of the grounds of rejection set forth by the Examiner has been overcome, and that the application is in condition for allowance. Such allowance is solicited.

Respectfully submitted,

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